2021-01-01

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10.1080/26388081.2020.1858447
Applied Phycology
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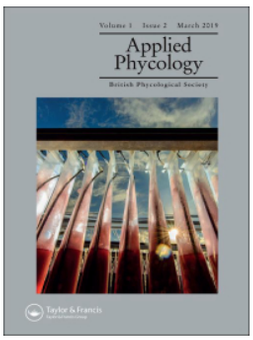

Applied Phycology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tapy20

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To cite this article: Barry Pettifor, Lina M. Rasmusson & Jason M. Hall-Spencer (2021) Dynamic structural colour increases photosynthetic performance in the alga *Ericaria selaginoides*, Applied Phycology, 2:1, 31-40, DOI: 10.1080/26388081.2020.1858447

To link to this article: https://doi.org/10.1080/26388081.2020.1858447

APPLIED PHYCOLOGY
2021, VOL. 2, NO. 1, 31–40
https://doi.org/10.1080/26388081.2020.1858447

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Dynamic structural colour increases photosynthetic performance in the alga *Ericaria selaginoides*

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ABSTRACT

Structural colour occurs when nanoscale structures interfere with incident light transmission and reflect particular wavelengths. The brown alga *Ericaria selaginoides* (Fucales, Phaeophyceae) has an opalescent photonic crystal anatomy that creates the blue colour of this seaweed but which responds to light, leading to speculation that the crystals have a photosynthetic role by modifying light transmission. Here, we characterize the colour response of *E. selaginoides* using time-lapse photography to capture responses to light treatments of different timing, duration, intensity and spectrum. The amount of light drove the colour response, with the most intense blue found in the dark which reduced rapidly in strong light and was not spectrum dependent. Chlorophyll *a* fluorometry showed that the maximum quantum efficiency of photosynthesis coincided with strong blue colouration but was reduced over two to three hours of illumination. This supports opalescent photonic crystals having a photosynthetic role by regulating light transmission to chloroplasts. Studies such as this could be used to improve solar cell efficiency and increase crop yield through the use of bio-inspired self-tuning mechanisms to optimize light transmission.

ARTICLE HISTORY
Received 10 September 2020
Accepted 28 November 2020

KEYWORDS
Biofuel; biomimetics; PAM fluorometry; photonic crystals; solar power

Introduction

Marine algae have evolved mechanisms to cope with rapid changes in light intensity and spectrum. Some have nanoscale structures for managing ambient irradiance which often reflect light creating "structural colour" from constructive or destructive interference (Prum, Quinn, & Torres, 2006). When the perceived colour of an object changes with viewing angle this is called iridescence (Doucet & Meadows, 2009) and reaches remarkable levels in the "rainbow wrack", *Ericaria selaginoides* (formerly Cystoseira tamariscifolia, Molinari Novoa & Guiry, 2020). This seaweed changes colour as irradiance varies, suggesting a photosynthetic role (Lopez-Garcia et al., 2018).

Natural examples of structural colour have roles in inter- and intra-specific communication, predator/herbivore deterrence and mate attraction (Chandler et al., 2017). Iridescence in nature is often striking, with bright, metallic or pearlescent qualities that are used in signalling (Vukusic et al., 1999). For example, tulip flowers have nano-scale striations which reflect UV/blue light to attract and orientate pollinators (Whitney et al., 2009) and, beyond communication, giant clams have iridescent cells that distribute light to algal symbionts (Holt et al., 2014) as a means to optimize photosynthesis. Where light is limited many plants possess nanoscale structures which cause iridescence. Some *Begonia* species have iridescence caused by photonic crystals in their epithelia which enhances green and low light uptake, both adaptations to forest floor conditions (Jacobs et al., 2016). The moss *Selaginella willdenowii* has epithelial lamellae causing blue reflectance thought to concentrate light for its chloroplasts (Hebant & Lee, 1984). Some red algae have opalescent photonic crystals (OPCs), whilst others, such as *Chondrus crispus*, create colour from layered cuticle structures which reflect UV-blue light, suggesting a photoprotective role (Chandler et al., 2015). However, in brown algae only OPCs have been discovered so far, as for example in *E. selaginoides* which occurs in low shore pools and the shallow subtidal where light levels vary widely and quickly depending on solar irradiance, tidal state, turbidity and canopy overgrowth. Making best use of light is a key factor in seaweed inter-species competition (Sanchez, Fernandez, & Arrontes, 2005). In *E. selaginoides*, iridescent structural colour is induced by intracellular OPCs containing lipid nanospheres in a lattice, the arrangement of which changes in response to light levels (Lopez-Garcia et al., 2018). In daylight OPCs disperse allowing light transmission through the thallus, but in darkness the nanospheres become close-packed and function as a lens, potentially

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Supplemental data for this article can be accessed here.

allowing them to regulate chloroplast irradiance (Lopez-Garcia et al., 2018).

Understanding how organisms manipulate light may be of great technological value and is receiving increased attention for example to increase energetic yields in solar panels or biofuel production. Finding ways to harvest more light energy may help reduce fossil fuel use by improving the design of photovoltaic cells, where inclusion of a single layer periodic nanostructure increased power conversion by 90% (Hsiao et al., 2011), with efforts ongoing to make further improvements using nanostructures (Cheng et al., 2017; Mahajan, Singh, & Arya, 2020). Where light is limited, bio-inspired light harvesting could increase photosynthesis and hence crop yield, for example using carbon nanoparticles (Swift et al., 2019). Research has also focussed on how to harness biology to make such structures, for example by using microbes (Parker & Townley, 2007). These applications address the fundamental human challenges of reducing fossil fuel use and food shortage, explaining the recent increase in iridescence research. Mimicking complex nanostructures such as the self-tuning mechanisms in *E. selaginoides* remains challenging (Onelli, Wilts, & Vignolini, 2018) and gaps remain in our ability to demonstrate that their modelled effects have the physiological effects postulated.

Based on modelled light harvesting by *E. selaginoides* (Lopez-Garcia et al., 2018), we assessed whether this species uses dynamic structural colour to optimize photosynthesis in low light conditions. We assessed the colour dynamics of these algae in terms of light spectrum, diel timing, period-length and irradiance levels. Moreover, we tested whether opalescent photonic crystals in *E. selaginoides* confer a photosynthetic advantage by measuring photosynthetic efficiency in iridescent and non-iridescent states, compared to a non-iridescent alga.

Materials & methods

Specimen collection and locations

Samples of *Ericaria selaginoides* and *Cystoseira foeniculacea* were collected between 2 July and 28 September 2019 from lower rocky shores in Cornwall, United Kingdom (New Polzeath, Falmouth and Hannafore Point). *Cystoseira foeniculacea* occurred with *E. selaginoides* at the collection sites and was chosen as a closely related but non-iridescent comparison in tests of photosynthetic efficiency. A sample frond of between 5 cm and 15 cm was cut from each thallus and different locations on the shore were used on return visits to avoid repeat sampling of the same individuals. Thalli were transported in aerated seawater containers and within three hours were secured in place in the 16°C controlled temperature (CT) aquaria described below.

Light conditions at a collection site

To record the range of light levels experienced by *E. selaginoides* a HOBO pendant light logger (Onset Computer Corporation, U.S.A.) was fixed for 24 hours to rock substratum at Polzeath from midday on 1 July 2019 in an area with abundant *E. selaginoides*. There was cloud cover on the first day and sunny conditions on the second day (Supplementary Figure S1). The HOBO logger recorded lux, converted to photosynthetically active radiation (PAR) by division by 47.7 determined from simultaneous measurements of lux and PAR in full sunlight to establish a midday summer full-sun benchmark and illustrate relative changes. Tide data were obtained using Tide Plotter v. 5.8, (Belfield Software Ltd., U.K.) and sunrise/sunset data sourced online from Timeanddate.com (2019).

Aquaria set up for specimen storage

Specimens were stored in a 10 l polycarbonate tank with constant aeration in a CT room set at 16°C (temperature similar to in-situ conditions at collection sites). The seawater was taken from Plymouth Sound, stored in the dark and filtered using wound polypropylene fibres initially at 10 μm and at 1 μm at the point of use (Wrekin Water Filtration Ltd., U.K.). Salinity was 33 psu, water was changed completely every two or three days and no nutrients were added. Light was provided using cool white 24 W 4000 K fluorescent tubes (British Electric Lighting Ltd., U.K.) on a 12:12 light:dark cycle. These delivered PAR at 330 μmol photons m^{-2} s^{-1} to the algal thalli, as measured with a SKP200 PAR meter (Skye Instruments Ltd., U.K.).

Photographic, microscopic and confocal fluorescence imaging

Unmagnified images were taken with a Canon 60D digital SLR through an EFS 18–55 mm USM lens (Canon Inc., Japan). Low power microscope images used a Nikon SMZ 660 (Nikon Instruments Europe B. V., Netherlands) and an iPhone7plus camera (Apple Inc., U.S.A.). High magnification optical images were taken through a MX4300H biological microscope (Meiji Techno, U.K.) using a 5Mpx Digi-Pad Camera (Medline Ceti, U.K.). For confocal fluorescence microscopy Sigma-Aldrich N3013 Nile red, a lipophilic stain,

(Merck, U.K.), was prepared to 0.314 μM using 30 kDa filtered seawater. Freshly cut samples of iridescent new growth were stained for 60 minutes without fixing or washing. Confocal images were acquired using a Zeiss 510 META (Carl Zeiss Microscopy GmbH, Germany) with excitation at 488 nm and detection on two channels: a KP685 chlorophyll filter and a bandpass 565–615 nm filter for the Nile red signal. The confocal image used was built with maximum intensity projections of six sections at 1 μm z-resolution with Fiji (a distribution of ImageJ, version. 2.0.0, Schindelin et al., 2012).

Controlling and measuring colour dynamics

To study changes in iridescence algae were held in a 10 l glass tank within a wooden dark-box (dimensions – w56 x d56 x h40 cm) at 16°C with a circulation pump. Specimens were weighted into place against a black background. Light within the dark-box was provided by an Aquabeam Ultra marine white LED panel (Aquaray, U.K.) placed on a polycarbonate sheet covering the tank. All lighting and the Canon DSLR camera were accommodated within the dark box which excluded all external light. Camera settings were fixed manually for focus, aperture (f20) and white balance. All photographs were taken with the aquarium lights off with illumination provided by a 15 W white LED photo light. Lighting changes and image capture were orchestrated by Python scripts running on a Raspberry Pi model B+ and monitored using a HOBO light logger with PAR levels measured for each treatment. Photographs were taken every 15 minutes and all images and HOBO data from each run were stored for analysis. The blue iridescence of thalli was measured as blue intensity expressed as a proportion of total red/green/blue (rgb) intensity using the colour histogram function of Fiji (a distribution of ImageJ, version. 2.0.0. Schindelin et al., 2012). Fiji results were transcribed into Excel (Microsoft Corp., U.S.A.) for analysis.

The response of blue colouration to light change events (light to dark or "LD"; dark to light, or "DL") was achieved by programming the Aquaray controller with the desired pattern of illumination and running Python code to capture images every 15 minutes. The core set of experiments studied response to 39 light change events with acclimation to the starting state (light or dark) for at least 4 hours. Blue colour change in response to variations in PAR level and event time of day was measured after 3 hours. Within the core experiment, but as an extreme case, three specimens were examined for 36 hours in continuous light conditions (LC) then 36 hours continuous dark conditions (DC).

An additional experiment used red, blue and green light treatments (means of two replicates) which involved placing coloured theatre lighting gels under the Aquabeam light panel. The resultant spectra were analysed with a SR9910.v7 spectroradiometer (Irradian Ltd., U.K.). A further test was performed of extremely reduced PAR, each period of low irradiance being interspersed with a period in full light to ensure that responses to low light were not confounded by a cumulative depletion of stored energy.

For initial trials of reduced PAR treatments the Aquaray controller was set to deliver reduced irradiance. The controller employs pulse width modulation (PWM) which involves rapidly turning full LEDs on and off, varying the "on" duration to achieve a dimming effect. Whilst this is perceived as dimmed light by the human eye the "on" pulses are actually at full intensity as was revealed by the HOBO logger which has a very fast sampling rate. Flashes of light, for example from surface waves or periodic breaks in sun-shading by macroalgae, are known to affect photosynthesis (Phillips & Myers, 1954) and so PWM may not be a satisfactory mimic of natural daylight gradients. To overcome this concern neutral density photographic filters were used instead.

Photosynthetic efficiency measurements

Pulse Amplitude Modulated (PAM) chlorophyll fluorometry was chosen as a quick, non-invasive method of measuring photosynthetic efficiency. The maximum photosynthetic performance of photosystem II (PSII) was measured using a JUNIOR-PAM device (Walz GmbH, Germany) managed through WinControl V3.29 software (Walz GmbH, Germany). A blue LED (450 nm) provided both measuring and actinic light sources. Measurement pulses were at 5 and 100 Hz., actinic light at 1,500 μmol photons m^{-2} s^{-1} and saturating pulses at 10,000 μmol photons m^{-2} s^{-1}. Neither species studied has the laminar leaf morphology for which Walz leaf-clips are designed so a set of holders were made from steel washers filled with epoxy resin. A central 3 mm hole was drilled and one face sealed with clear plastic to create a circular well 1.5 mm deep. Ramuli (the short spike-like branches arising from the main stem) were dissected from thalli and laid side by side within the well of the holder. This enabled samples to be placed into the magnetic leaf clip so that the light guide faced epithelium at a consistent 90° angle. Care was taken to ensure that samples remained wet by pipetting seawater into the holder.

Maximum (Fm) and minimum (Fo) fluorescence were measured with the PAM fluorometer and variable fluorescence (Fv) derived as Fm–Fo. The maximum quantum yield of PSII as given by Fv/Fm is an indicator

of the efficiency of PSII photochemistry (Maxwell & Johnson, 2000). Samples were given treatments in either LC in an aquarium storage tank or in DC by covering with a larger, inverted tank masked with thick aluminium foil. Each thallus was split and given DC overnight then one of each pair received LC treatment for between two and 5 hours before Fv/Fm was measured for both. LC samples were dark-adapted for 10 minutes before measuring Fv/Fm. Although 15 minutes dark adaptation is seen as standard 10 minutes was regarded as satisfactory for testing this species as only five minutes has been shown to give results consistent with much longer dark treatments (Celis-Pla et al., 2014a). Fv/Fm of *E. selaginoides* was measured in DC and LC for 24 discrete biological individuals. Tests were performed in triplicate and averaged to determine the Fv/Fm for each individual thallus in LC and DC (each individual was therefore measured 6 times; 3 in DC and 3 in LC).

Statistical analysis

Linear regression was used to assess the correlation of PAR and treatment duration. A one-way ANOVA was performed to assess if time of event (using four time bins of six hours) affected colouration response with homoscedasticity of variance being tested with Levene's test (Levene, 1960). The rates of colouration change between treatment and Fv/Fm values (and logs thereof) were not normally distributed according to Shapiro-Wilks tests so differences in means were assessed with Wilcoxon rank sum tests. Hourly means of Fv/Fm were tested by one-way ANOVA after testing homoscedasticity of variance with a Levene's test. Differences were regarded as significant at p values above 0.05. All statistical tests were performed in R via R Studio (R Core Team, 2018).

Results

Light levels on the rocky shore

Light logging for 24 hours over two tidal cycles showed that low-shore *Ericaria selaginoides* live in conditions where light can vary from PAR of 1,800 µmol photons m^{-2} s^{-1} to zero (Supplementary Figure S1). There were 11 hours when PAR was above zero but lower than 100 µmol photons m^{-2} s^{-1}, including 3 hours with PAR lower than 10 µmol photons m^{-2} s^{-1}. During the morning of the second day, PAR increased very rapidly to 1,500 µmol photons m^{-2} s^{-1} within just 30 minutes as the tide fell below the logger (Supplementary Figure S1).

Morphology and cellular structure

Figure 1 shows the morphology of *E. selaginoides* at increasing magnification. A typical thallus showing blue iridescence is seen in Figure 1(a) and under low power magnification in Figure 1(b). The blue colouration is seen to derive from numerous points corresponding to OPCs (Figure 1(c)). The position of OPC vesicles in relation to chloroplasts is seen in Figure 1(d) and shows that some OPCs are bright whilst others are more diffuse.

Colour dynamics

Blue iridescence changed reversibly on transition between dark (DC) and light (LC) conditions whereby the blue intensity was greatest in DC and reduced in LC. A detailed example of colour response at PAR of 330 µmol photons m^{-2} s^{-1} is shown in Figure 2. This example was regarded as typical and similar changes were observed across 39 events where the light regime was changed from dark to light (DL) and vice-versa (LD), and in all cases the blue colouration became stronger when transitioning to dark and diminished on transition to light (Supplementary Figure S2). The blue response occurred consistently at all PAR levels above 45 µmol photons m^{-2} s^{-1} (Supplementary Figure S3) with no difference observed with regard to the time of day light change events occurred (ANOVA of 6 hour time slots $p = 0.153$, Supplementary Table S1). For three specimens examined for 36 hours in continuous LC followed by 36 hours continuous DC the blue colouration change was consistent with expectations and sustained (results not shown). There was a significant tendency for blue colouration to continue changing in longer treatment durations, reducing in light exposure ($R^2 = 0.17$, $p = 0.037$, $n = 21$) and it appeared to increase in dark treatment although not statistically significantly so in this experiment ($R^2 = 0.13$, $p = 0.0799$, $n = 18$; Supplementary Figure S4).

The blue colouration response was tested using red, blue and green filters. Each filter reduced the effective PAR to 70 µmol photons m^{-2} s^{-1} but responses were similar to those in full spectrum light regardless of filter colour (Supplementary Figure S5).

The response to four lower irradiance treatments (60, 28, 12 and 3 µmol photons m^{-2} s^{-1}) was tested. Even at the lowest PAR a response was apparent but in those below 28 µmol photons m^{-2} s^{-1} the initial change to blue colouration reversed quickly (Figure 3).

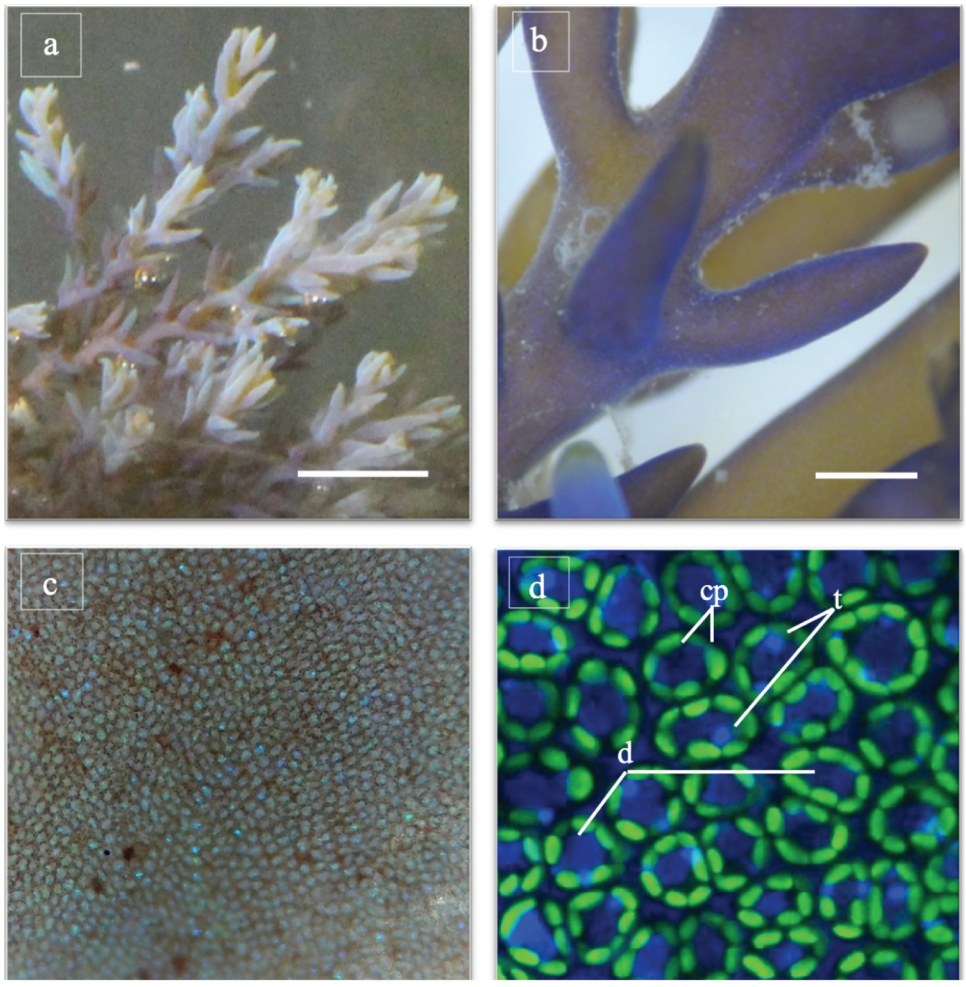

Figure 1. *Ericaria selaginoides* morphology (a) after 6 h darkness showing blue iridescence (scale bar 1 cm). (b) iridescent algal surface (scale 2 mm). (c) cells with blue points corresponding to opalescent photonic crystals (OPCs)(scale 100 μm). (d) confocal image of chloroplasts in green (cp) and OPCs in blue in tight lattice (t) or diffused (d) state (scale 15 μm).

Photosynthetic efficiency

Fv/Fm of *E. selaginoides* was measured in 24 discrete biological individuals. The mean Fv/Fm for specimens in DC was 0.711 and 0.676 in LC ($n = 24$, $W = 470$, $p < 0.001$, Figure 4) indicating a modest but significant increase in the mean maximum photochemical efficiency in DC of 5.2%. The mean Fv/Fm for the non-iridescent *C. foeniculacea* in DC was 0.713 and in LC 0.719 ($n = 4$, $W = 6.5$, $p = 0.772$, Figure 4), indicating no difference between treatments.

Given that the colour response typically took over 3 h to complete, Fv/Fm was also tested at intervals for five hours after all-night DC treatment. Nine specimens were measured in triplicate and averaged into 1hour bins. In all cases Fv/Fm fell to a low point between 2 to 3 hours after DC and then recovered (Figure 5).

Discussion

We found that blue iridescence in *Ericaria selaginoides* is responsive to low irradiance, independent of light spectrum and time of day. Photosynthetic performance was highest in the blue dark-adapted state whilst the non-iridescent *Cystoseira foeniculacea* was unresponsive to the same treatment.

Our model iridescent alga is exposed to extremes of irradiance in nature with extended periods of low light as well as extremely rapid increases in light. For intertidal algae these conditions present a dual challenge: the need to harvest sufficient light whilst risking damage from sudden increases in irradiance (Falkowski & Raven, 2007). Dynamic nanostructures may address these challenges by focussing more light on chloroplasts in the bluer state found in darker conditions and dissipating energy by transmission or reflection in strongly lit conditions (Lopez-Garcia et al., 2018). We have shown that blue, dark treated thalli had the highest photochemical efficiency, dropping to a minimum after 2 to 3 h of light and then partially recovering. This was broadly coincident with the period over which the blue colouration changes and light gradients experienced at sunrise/sunset or during immersion/

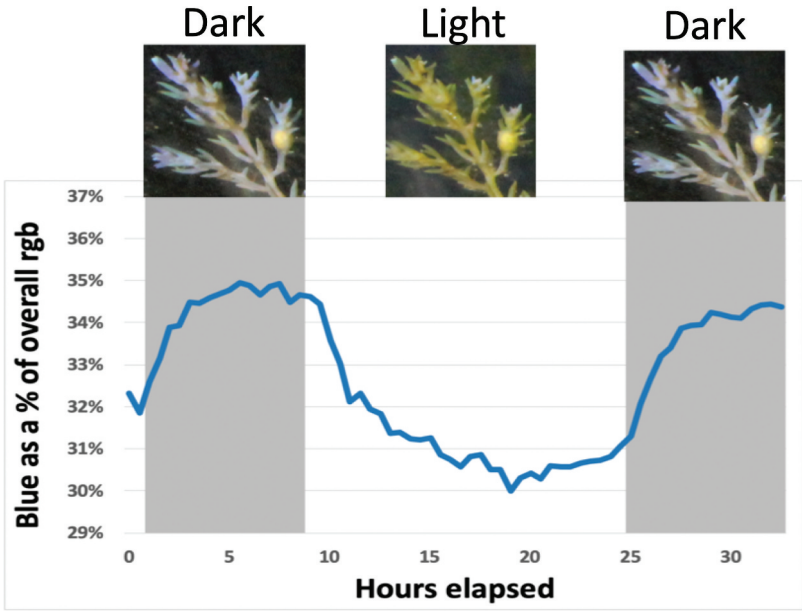

Figure 2. Response of *Ericaria selaginoides* blue iridescence to periods of dark conditions (DC, shaded grey) and light conditions (LC, white) over 32 h. Light treatment was at PAR of 330 µmol photons m^{-2} $s^{-1.}$ The line indicates blue intensity as a proportion of overall intensity (being the sum of red, green and blue channels or 'rgb'). Photographs illustrate the peak and minimal blue iridescence of the specimen tested.

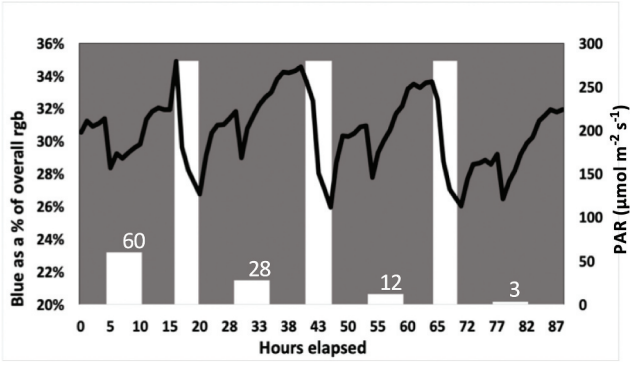

Figure 3. *Ericaria selaginoides* blue iridescence response to four reduced PAR levels ranging from 60 to 3 µmol photons m^{-2} $s^{-1,}$ in each case interspersed with periods of full light at 280 µmol photons m^{-2} s^{-1}. Black line shows mean blue intensity as a proportion of overall intensity (being the sum of red, green and blue channels or 'rgb') averaged for two specimens. White bars indicate periods of illumination and the PAR intensity of each, and grey shading denotes dark conditions.

emersion. By contrast, the non-iridescent *C. foeniculacea* showed no response in terms of Fv/Fm between light and dark treatments further supporting the hypothesis that the mechanism driving iridescence may also regulate photosynthesis.

Studies which link nanostructures to improved photosynthetic performance are rare. Some species of the genus *Begonia*, a shady forest floor specialist, have photonic crystals formed by regular thylakoid spacing which increased the quantum yield of PSII by some 10–15% under low light (Jacobs et al., 2016). The same study also observed a reduction in photosynthetic efficiency with increasing light levels, likely connected with limitations in electron transport downstream of PSII. In a study of the non-iridescent *Selaginella erythropus* photonic multilayers create a subtle blue reflection which was not easily observed suggesting that nanostructures may exist in other plants but have gone unnoticed (Masters et al., 2018). We have assumed that *C. foeniculacea* does not have nanostructures that regulate light transmission, but they may exist without creating visible iridescence.

In almost all sunlit ecosystems a midday suppression of photosynthesis has been observed (Falkowski & Raven, 2007) as seen for example in *Ulva lactuca* (Longstaff et al., 2002) which can persist in continuous light treatments suggesting an endogenous diel clock is operating as also seen in some phytoplankton (Harding et al., 1981). We found that this was not the case for *E. selaginoides* given that continuous 36 hours dark and light treatments elicited no unexpected response in blue colouration. Whilst the time of day appears not to affect the propensity to respond there may be a signal that the extent of response is greater when a light change occurs between 06:00 and 12:00 (Supplementary Table S1) which coincides with dawn to full sun. At the time of study, *Cystoseira foeniculacea* did show a drop in photosynthetic efficiency after approximately 4 hourswhich may reflect a midday suppression triggered by light or by an internal clock.

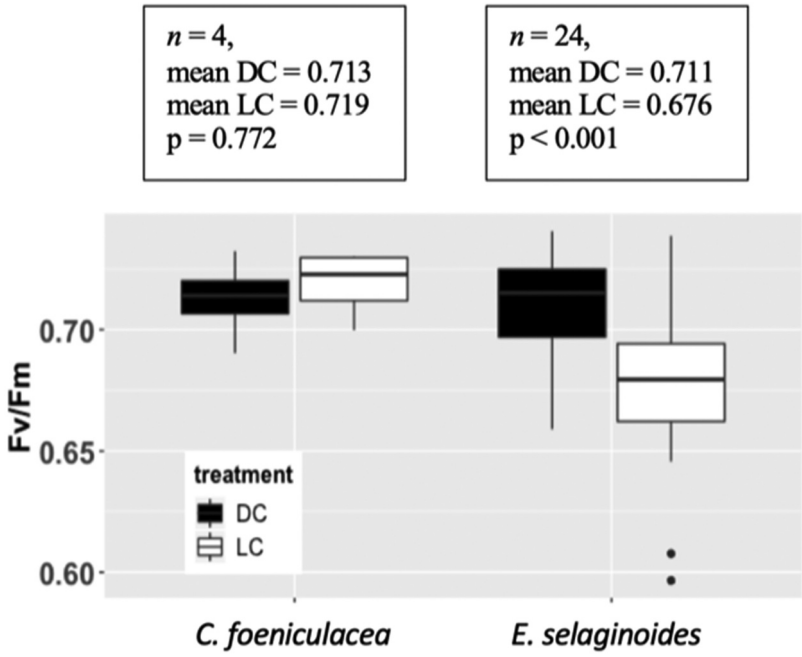

Figure 4. Maximum photochemical efficiency measured as Fv/Fm for *Cystoseira foeniculacea* and *Ericaria selaginoides* after dark treatment (DC) overnight or after light (LC) treatment for between 2 and 5 hours.

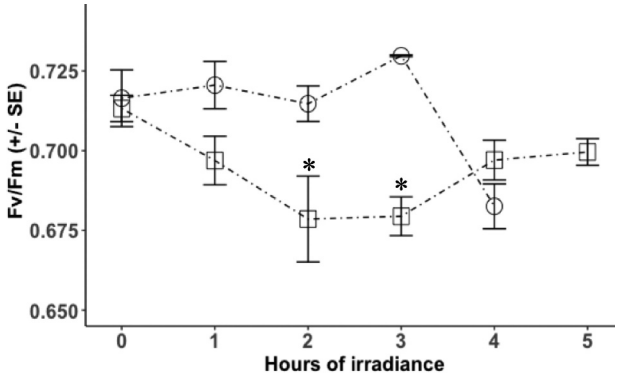

Figure 5. Maximum photochemical efficiency (Fv/Fm) of nine specimens of *Ericaria selaginoides* (squares) and three specimens of *Cystoseira foeniculacea* (circles) during illumination after overnight dark treatment. Each specimen was tested in triplicate and averaged. Measures were repeated over a 5hour period and show reduced Fv/Fm between 2 to 3 hours after illumination started. Mean Fv/Fm for *E. selaginoides* is significantly different from time zero values at 2 and 3 hours (* denotes $p < 0.05$).

Seaweeds may be compromised by strong light such as at midday and the loss of blue colouration could be a photoprotective mechanism synchronized with relatively slow increases in irradiance at sunrise and/or upon emersion. Supra-optimal irradiance, especially UV, can impair photosynthesis by damaging PSII reaction centres and DNA (Gomez & Huovinen, 2010; Larkum & Wood, 1993), or by generating reactive oxygen species (ROS) harmful to chloroplast integrity and function (Malanga, Calmanovici, & Puntarulo, 1997). Dispersal of the nanospheres (coincident with loss of perceived blue colouration) allows more light to be transmitted rather than being refracted towards chloroplasts (Lopez-Garcia et al., 2018) hence attenuating chloroplast irradiance across the spectrum. However, the blue state exacerbates the risk that a sudden increase in irradiance, for example a sunny period on a cloudy day, damages the photosystem. One possible explanation for the recovery in Fv/Fm after two to three hours is that in the blue state photoinhibition is in place but is relaxed once the OPC nanospheres have dispersed sufficiently to provide optical protection. Our observations of *E. selaginoides* are likely part of a suite of light management tools including optical optimization, photoinhibition and photoprotective mechanisms. For example, *E. selaginoides* from shallow water was photo-inhibited in full light but this relaxed when transplanted to lower light (Celis-Pla et al., 2014b). In addition, phenolic compounds, which rely on nutrient availability, are exuded as UV protection as seen in *E. selaginoides* during high irradiance on a diurnal cycle (Abdala-Diaz et al., 2006). Furthermore, blue OPCs reflect light in the UV/blue spectrum (Lopez-Garcia et al., 2018) and so may provide a degree of protection against the most harmful UV and high energy light even when light harvesting is maximized, as appears to be the case in iridescent *Chondrus crispus* (Chandler et al., 2015). Using excess electrons to create ROS may allow more energy to be used photochemically in PSII but at the cost of cell damage

and/or investment in scavenging molecules (Cruces et al., 2019). Therefore, the ultimate advantage from dynamic OPCs for *E. selaginoides* may be savings in resources for tuning and repair of antenna systems, producing phenolic photoprotection or ROS scavenging compounds.

Lopez-Garcia et al. (2018) showed that the blue iridescence is dynamic, and the present study has confirmed their results and builds on them by further characterizing the dynamic blue response to light changes. The full spectrum response was repeated using coloured filters which showed changes consistent with those in white light and the extent of change remained at approximately a four percentage points swing in blue/rgb. Light is known to be a physiological trigger, for example in fucoid gamete release (Pearson, Serrao, & Brawley, 1998), and blue-shift is a means of detecting immersion (Pearson et al., 2004). The iridescence dynamics of *E. selaginoides* appear to respond to the incidence of photons regardless of spectrum which suggests a relevance beyond detecting immersion. The response to progressively lower irradiance confirms that OPCs change configuration at PAR as low as 3 μmol photons m^{-2} s^{-1}, albeit the response was muted and returned to blue quickly. This gives further weight to the suggestion that the OPC configuration changes are relevant in low light but it seems that light must remain above a given level in order to sustain the change. One interpretation of this pattern is that the OPC anticipates the gradient of a light increase (at dawn or on emersion) by commencing the nanosphere reconfiguration as soon as a change is detected because it will take some time to complete. In 3 μmol photons m^{-2} s^{-1} however a reversion to blue state occured rapidly, effectively aborting the change. These results were obtained with neutral density filters and the original experiments with PWM dimming did not show this tentative response. PWM is known to affect plant physiology such that pulsed light results in more growth than equivalent amounts of continuous light (Philips & Myers, 1954; Shimada & Taniguchi, 2011) and therefore may have been sensed as being equivalent to stronger light. The partial change in state seen in the OPCs in the confocal image may have occurred because the intense laser light had triggered the process of change between the iridescent and non-iridescent state for some OPCs, a process which can occur within seconds in unnaturally intense light (Lopez-Garcia et al., 2018), further supporting the observation that iridescence response is proportional to photon flux and hence may have a photoprotective role.

Continued research is justified to better understand the sensing and mechanics of OPC physiology given its high biomimetic value. The application of photonic crystals in increasing photon:current ratios has been known for some time (Hsiao et al., 2011; Mihi et al., 2008). OPCs implemented as a controllable lens would open up numerous possibilities to enable solar cells to become self-tuning to light at different photon flux, spectrum or direction. OPCs could also be used to create paints, textiles and artwork which respond to external stimuli (Schenk, 2015) or improve crop yields (Swift et al., 2019).

In conclusion, we have confirmed the dynamic structural colour response to light and dark observed by a previous study and extended the knowledge of the response to lower light, eliminating spectrum shift and diel clocks as drivers. The response is triggered by, and seems proportional to, photon flux suggesting that the colour response is a reaction to increasing irradiance resulting in enhanced light harvesting and/or photoprotection. Moreover, an association has been discovered between photosynthetic efficiency and dynamic colour. *Ericaria selaginoides* remains the only example we are aware of in which dynamic structural colour in a plant or alga has a documented photosynthetic role.

Author contributions

BP designed the study, analysed the results and wrote the article with guidance from LMR and JMH-S who both also commented on the drafts and final version.

Acknowledgments

The authors are indebted to University of Plymouth staff: Dr Ben Ciotti for statistical advice, Andrew Atfield for technical support and to Dr Jo Triner and Jane Thorning for help with optical imaging. We are grateful to Davis Laundon of the Marine Biological Association for assistance with confocal imaging. We also thank Professor Juliet Brodie, Dr Ilse-Christine Gebeshuber, Dr Martin Lopez-Garcia and Dr Nathan Masters for helpful discussions.

Data availability statement

All the data required to review the conclusions are included within the paper or Supporting Information. Further information is available from Barry Pettifor on request.

Disclosure statement

The authors declare that they have no conflicts of interest.

Funding

This work was supported by the University of Plymouth (BP & JMH-S) and the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Stockholm) under Grant number 2017-00363_Formas (LMR).

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